Fig. S1

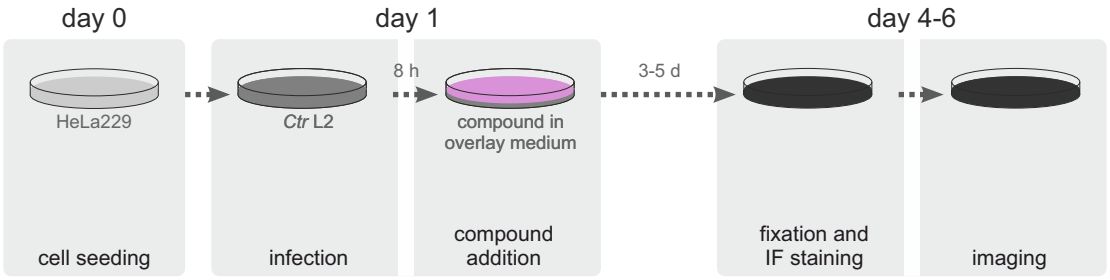

Figure S1. Workflow scheme of liquid overlay medium-based plaque assay. Experimental time-scale of the plaque assay including cell seeding, infection, addition of liquid overlay medium, incubation and final immunofluorescence staining.

Fig. S2

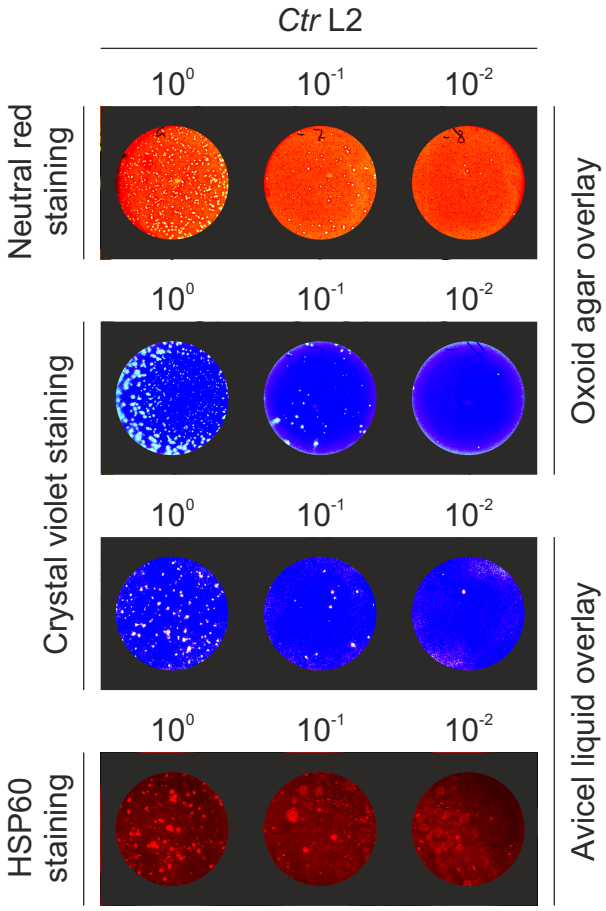

Figure S2. Comparison of different plaque assays and staining protocols for the titration of *C. trachomatis* L2. Images comparing the titration of *C. trachomatis* L2 (*Ctr* L2) plaques in a McCoy cell monolayer using different overlay media and different staining protocols (see materials and methods section for experimental details). Numbers indicate dilution factor of bacteria.

Fig. S3

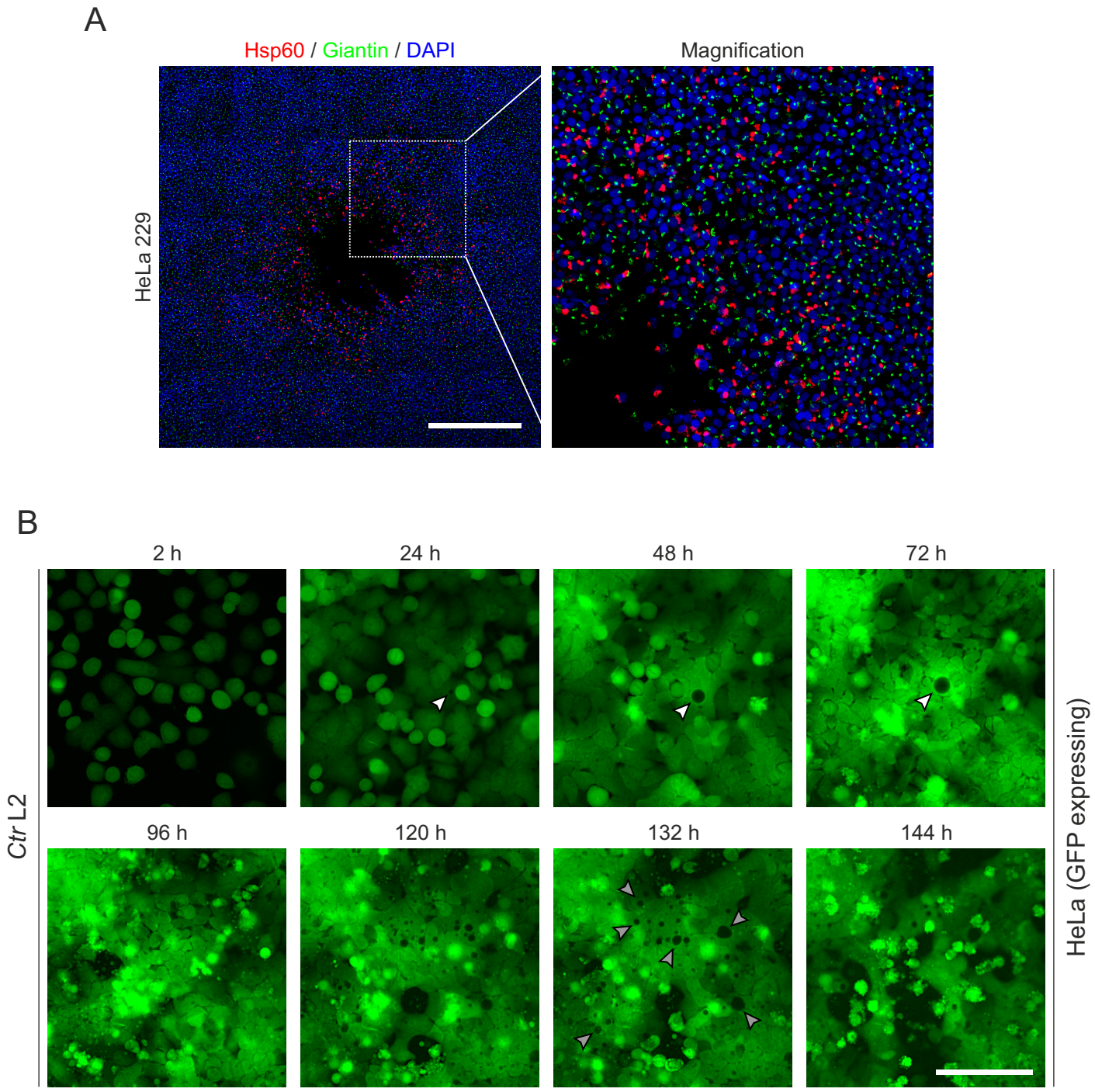

Figure S3. Microscopic analysis of plaque morphology and formation. (A) Immunofluorescence images showing detailed morphology of a *C. muridarum* plaque in a HeLa 229 cell monolayer. Cells were stained with antibodies against chlamydial Hsp60 (red) and Giantin (green), DNA was stained with DAPI (blue). Scale bar, 100 µm. **(B)** Fluorescence images showing a monolayer of GFP-expressing HeLa cells infected with *C. trachomatis* L2 (*Ctr* L2) for the indicated time. A primary inclusion is marked with a white arrow. At later time points, multiple inclusions originating from the primary inclusion can be detected (gray arrows). Images were taken from a time-lapse movie. Scale bar, 50 µm.